The Influence of Harvesting Period on Lipid Associated Antioxidant Activity of Semicontinuously Grown *Chlorella vulgaris*

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Abstract

A green alga, *Chlorella vulgaris* was grown semi-continuosly at various harvesting periods, and the lipid content and its associated antioxidant activity was examined. The harvesting periods were 9, 18, and 27 days, while the algal culture was placed in 10 L glass bottles provided with aeration for gas exchange and mixing. Light source was obtained from 4×40 watt cool fluorescent lamps placed at one side of the culture. Room temperature was 26-32°C. Algal lipid extraction was done based on liquid phase separation of methanol:chloroform:water and the antioxidant activity was examined by means of oxidation inhibitory in ethanol emulsified limnoleic acid. In addition, a TLC analysis was performed to identify the antioxidant compounds soluble in the lipid. The results showed that harvesting period had a significant influence on the algal lipid content, which were 11.94, 12.96, and 16.51% of the dry weight in the culture with harvesting periods of 9, 18, and 27 days, respectively. No remarkable effect of the harvesting period on the antioxidant activity, which were observed to inhibit oxidation of linoleic acid up to 67-71%. There were five compounds found can be associated with the algal antioxidant activity, which were pheophorbide-a, chlorophyll-b, chlorophyll-a, phaeophytin-a, β-carotene, and an unidentified one.

Key words: microalgae, *Chlorella vulgaris*, lipid, antioxidant, harvesting period

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Introduction

Indonesian water has a very large biodiversity of microalgae (Chrismadha & Ali, 2007; Chrismadha & Lukman, 2008; Sulawesty & Satya, 2008), which are known as one of the potential organisms useful in various ways, such as for food, feed, fuel, medicine, industry and in controlling pollution (Banerjee *et al.,* 2002; Berberoglu *et al.,* 2006, Venkatesan *et al.,* 2006; Carlsson *et al.,* 2007). Microalgae are also rich sources of several fine chemicals of economic value such as vitamins, carotenoids, phycobiliprotein, fatty acids, *etc.* with varied properties and possess as anti-inflammatory, anticancer, antifungal, antioxidants and immune-modulators agents (Borowitzka, 1988a; Chrismadha & Borowitzka, 1994; Reddi *et al.,* 2000*;* Wen & Chen, 2003; Benedetti *et al.,* 2004; El-Baky *et al.,* 2004; Wu *et al.,* 2005; Chrismadha *et al.,* 2006; Lee *et al.,* 2010).

This paper reports an evaluation on the antioxidant properties of locally isolated microalgae, *Chlorella vulgaris*. Antioxidant productivity from microalgal culture is not merely depends on the antioxidant content in the algal biomass, as has been previously reported for fatty acid production from *Phaeodactylum tricornutum* and *Spirulina* sp. cultures (Sukenik, 1991; Chrismadha & Borowitzka, 1994) that the fatty acid productivity was the function of the biomass productivity and its fatty acid content. Similarly, the antioxidant production from *C. vulgaris* culture can be assumed to be dependence on the biomass productivity and the antioxidant content.

Harvesting period is one among the microalgal biomass productivity factors that up to the recent time receive less attention, even though this parameter largely determine both the quantity and quality of the biomasss, as it is directly related to the culture growth phase. Shorter harvesting period generally

maintains the algal culture in the exponential state with higher growth rate but lower culture density (Chrismadha, 2007). In the mean time, longer harvesting period tends to bring the culture into stationary phase condition with higher cell density but lower growth rate. Chrismadha & Borowitzka (1994) reported that the biomass productivity of semi continuously grown *P. tricornutum* culture can be maximized by optimizing the culture density. Meanwhile, Vonshak & Richmond (1985) reported that 'self-shading effect' phenomenon is due to the high culture density in race way open ponds which reduce the culture growth and productivity.

Growth phase has also widely known to influence the physiological state of the algal cells, including the biopigments and other biochemical properties. For an example, Piorreck & Pohl (1978) and Chrismadha (2007) has reported the decreasing in pigments content of various green and blue green algae with the culture development toward the stationary phase. Sukenik (1991) emphasized the importance to consider the content of targeting chemical during the harvesting period to attain its economic feasibility. Accordingly, the development of *C. vulgaris* culture for antioxidant production has to be directed to obtain optimum state of the biomass productivity and its chemicals content.

Materials and Methods

The green alga *C vulgaris* used in this experiment was obtained from microalgal collection of The Planktonology Laboratory of the Research Center for Limnology, Indonesian Institute of Sciences (LIPI) at Cibinong, Indonesia. The alga was grown semi continuosly at various harvesting periods in 10 L glass bottles provided with an air sparger for gas exchange and a magnetic stirrer bar for mixing. The cultures were placed in the culture room with daily temperature of 26-32°C. Light was provided from 4×40 watt white flourescent lamps placed at one side giving the average light intensity at the bottle surface of 3000 lux.

The media culture was based on PHM media (Borowitzka, 1988b) with innitial pH of 6.8-7.0. Inoculum was prepared from exponential grown 2 L culture, inserted into the experimental cultures of 8 L effective volume by about 40× dilution rate. After that, the cultures were allowed to grow and harvested regularly at various harvesting periods, which were 9, 18, and 27 days, respectively. The harvesting volume was 6 L which was drawn into the harvesting jar and replaced by new fresh media of the same volume.

Daily observation on the algal growth was carried out by means of culture cell density counted in a haematocytometer under a light microscope. The culture cell density data was then used to calculate the algal growth rate according to equation as follows:

$$\mu = \frac{\ln (x_t/x_o)}{t}$$

Where: μ = growth rate (cell division/day), x_t = cell density at day t, x_o = cell density at day 0, and t = time (days).

The algal biomass concentration was determined gravimetrically after overnight oven at 100°C, and based on the obtained data the culture productivity was calculated according to the following equation:

$$P = \frac{m_t - m_o}{t}$$

Where: P = culture productivity (g/L/day), m_t = biomass concentration at day t, m_o = biomass concentration at day 0, and t = time (days).

Chlorophyll content was examined according to APHA (1998). Algal lipid extraction was based on liquid phase separation of methanol : chloroform : water (Blight & Dyer, 1959) and the lipid content was determined gravimetrically. The antioxidant activity was examined by means of oxidation inhibitory in ethanol emulsified limnoleic acid (Ferric thiocyanate method; Kikuzaki & Nakatani, 1993; Endrini *et al.,* 2002; Aqil *et al.,* 2006). The examination was performed both to the algal biomass and the extracted lipid. In addition, a TLC analysis was performed to identify the antioxidant compounds soluble in the lipid (Stahl, 1969; Prangdimurti *et al.,* 2006).

Results and Discussion

This experiment shows a remarkable influence of harvesting period on the growth of *C. vulgaris*. The maximum cell density was achieved at 18 days harvesting period, which was 23.59×10^6 cells/ml, while at 9 and 27 days harvesting period the average cell density were almost equal, which were 19.17×10^6 and 19.20×10^6 cells/ml, respectively (Figure 1). In the mean time, a consistent drop of the algal specific growth rate with the harvesting period was observed, which were 1.74; 0.92; and 0.61 µ/day/L at harvesting periods of 9, 18, and 27 days, respectively. It was also observed that the harvesting period consistently increased the biomass concentration but resulted in lower the biomass productivity (Figure 2). The average biomass concentration of 9 days harvesting culture was 0.22 g/L, which increases to 0.35 and 0.37 g/L in the culture harvested every 18 and 27 days, respectively. In contrast, the culture productivity reduced from 0.025 g/L/day at 9 days harvesting period to 0.020 and 0.014 g/L/day at 18 and 27 days harvesting periods, respectively. A similar pattern has been reported in *Spirulina fusiformis* culture (Chrismadha, 2007), which was attributed to growth phase and self shading effect at high culture density. As has been known, shorter harvesting period maintain the algal culture in the exponential state with higher growth rate but lower culture density, meanwhile longer harvesting period bring the culture into stationary phase condition with higher cell density but lower growth rate. According to Vonshak & Richmond (1985) culture productivity is function of the growth rate and the culture density. The above result indicated that the increase in culture density at longer harvesting period was not able to compensate the reduction in growth rate, and give a consequency of lower productivity. At the same time, Vonshak & Richmond (1985) also reported that a self shading effect phenomenon occurred at high culture density which reduced the algal growth and productivity due to light limitation. A decrease in productivity of *P. tricornutum* culture with the cell density has also been reported (Chrismadha & Borowitzka, 1994).

The algal lipid contents were 11.94; 12.96; and 16.51% of the biomass in the culture with harvesting periods of 9, 18, and 27 days, respectively (Figure 3). Some reports on the increase in algal cell lipid content with growth phase have been established (Piorreck & Pohl, 1978; Chrismadha & Borowitzka, 1994). This current result confirms that the physiological state of the longer period harvesting culture resemble ones of the late growth phase, which in this case characterized by higher lipid content.

No remarkable effect of the harvesting period on the algal antioxidant activity, which was observed to inhibit oxidation of linoleic acid up to 67-71% (Figure 3). In the mean time, the extracted lipid had a slightly higher antioxidant activity compared to that of the algal biomass. This indicates that the antioxidant in the alga is largely associated with lipid compounds. It is in consistent with some studies which pointed out the antioxidant occurrence in algae mainly related to the lipophilic biopigments (Matsukawa *et al.* 2000; El-Baky *et al.,* 2004; Hu *et al.,* 2009). Result of the TLC analysis wass in an agreement with this. There were five identified compounds could be associated with the algal antioxidant activity, which were pheophorbidea, chlorophyll-b, chlorophyll-a, phaeophytin-a, β-carotene.

Tabel 1. Antioxidant associated compounds of *C. Vulgaris*

| Fraction	R_f	Color	Substances
1	0.2	grey-green	pheophorbide-a
2	0.31	yellow-green	chlorophyll-b
3	0.41	grey-green	un-identified
4	0.6	blue-green	chlorophyll-a
5	0,93	grey-green	phaeophytin-a
6	0,99	orange-yellow	β –caroten

Conclusion

The results showed that harvesting period has a significant influence on the algal lipid content, which were 11.94; 12.96; and 16.51% of the dry weight in the culture with harvesting periods of 9, 18, and 27 days, respectively. No remarkable effect of the harvesting period on the antioxidant activity, which were observed to inhibit oxidation of linoleic acid up to 67-71%. There were five compounds identified can be associated with the algal antioxidant activity, which were pheophorbide-a, chlorophyll-b, chlorophyll-a, phaeophytin-a, and β-carotene.
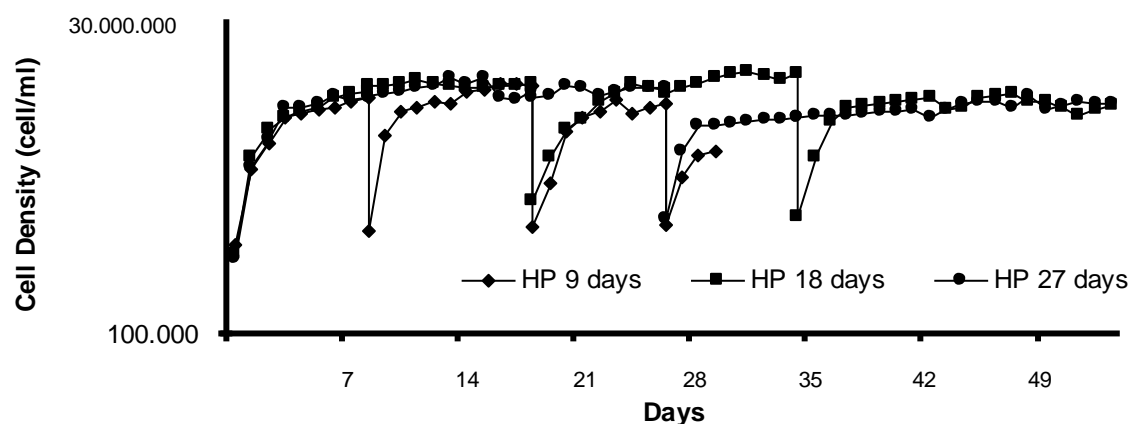
Figure 1. Cell density development of *C. vulgaris* culture under various harvesting periods
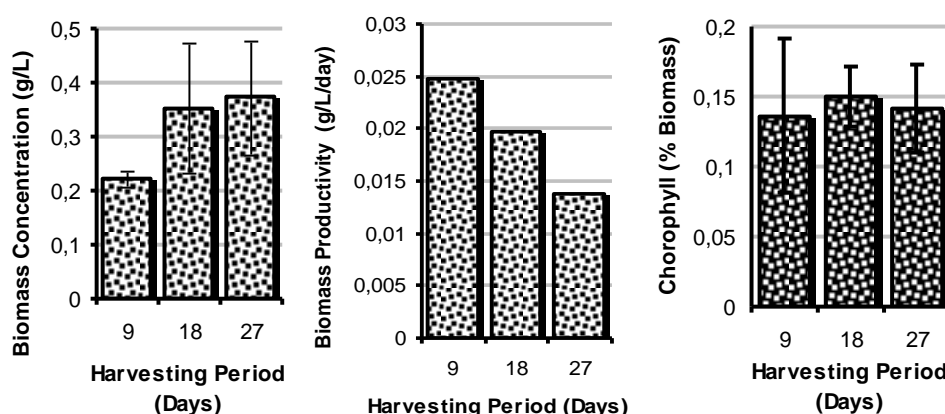
Figure 2. Biomass concentration, biomass productivity, and chlorophyll content of *C vulgaris* culture under various harvesting periods
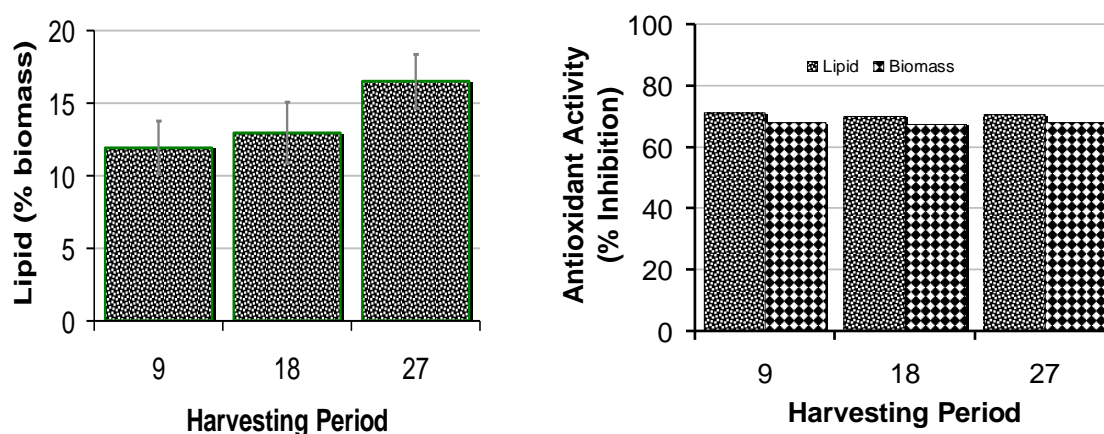
Figure 3. Lipid content and antioxidant activity of *C vulgaris* culture under various harvesting periods

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